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FEB. - 8 - 1899

[Reprinted from THE PHILADELPHIA MEDICAL JOURNAL, November 19, 1898.]

EXPERIMENTAL STUDIES ON THE ORIGIN OF THE  
CHARCOT-LEYDEN CRYSTALS, WITH AN AC-  
COUNT OF THEIR CHEMISTRY, OCCURRENCE AND  
DIAGNOSTIC IMPORTANCE.

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THE part played by the Charcot-Leyden crystals upon the medical stage has been a most varied one. Though at first regarded as mere pathologic curiosities, rarely seen and of no especial significance, later observations, by showing their tendency to occur frequently or even constantly in certain diseases and morbid conditions, have given them a much more important position and, in some cases, some diagnostic significance. In the present communication, I shall give the results of a series of experiments bearing upon the origin of these crystals, together with a brief but complete account of their occurrence, properties and importance.

The first definite notice taken of the crystals under consideration dates from their discovery, in 1853, by Charcot and Robin in the spleen of a patient who had died of leukemia. Probably they had been seen before; in fact, Zenker, in 1876, when interest in this subject had been greatly increased by the work of Neumann and Leyden, resurrected from the "darkness of his portfolio" a drawing and a description of crystals undoubtedly the same as those of Charcot, and from the same source, bearing the date 1851. Following the work of Charcot and Robin, there appeared many articles describing the finding of these crystals in the most diverse situations. Besides a rapidly growing list of their occurrence in the

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blood, marrow, spleen and other tissues in leukemia, and their demonstration by Neumann in 1869 in the normal bone-marrow in smaller quantities, there appeared from time to time observations of their presence in the sputum in various diseases of the lungs and bronchi, culminating in the work of Leyden, and his demonstration of their almost constant occurrence in the sputum in bronchial asthma, while Leichtenstern, Bizzozero, and others have found them in many diseases of the intestines caused by different varieties of the entozoa. More recently Lewy has shown that they occur in the majority of nasal polyps and papillomata. In addition to these conditions in which they are seen very frequently, observations of their occurrence in the most unexpected situations have been made; in 1854 by Förster in a mucoid tumor of the eye, and in the thickened mucous membrane of a dilated biliary duct; in 1862 by Wagner in a thrombus in the portal vein of a woman who had died of puerperal sepsis; by Eichhorst in a purulent pleural exudate, and by Lewy in a carcinoma of the cervix; while Salkowski states that the crystals obtained by Zahn in artificial thrombi in frogs, and by Brondgeest from frozen frogs' blood, are identical with those under consideration.

Practically the same description of the crystals is given by all observers. They are long drawn-out double pyramids, with flat sides and sharp angles, colorless, exceedingly brittle, of varying size and most resistant to putrefying processes. As to the crystallographic system to which they belong, they have been variously regarded as quadratic octohedra, rhombic plates and monoclinic forms, though the work of Theodore Cohn seems to show beyond doubt that they are double hexagonal pyramids. The crystals are insoluble in cold water, alcohol, ether, xylol, chloroform and creosote; soluble in almost all acids and alkalies, and they show

a marked affinity for the eosin and acid-fuchsin stains. Besides their common designation of Charcot-Leyden crystals, they have appeared under the names of Robin, Vulpian, Neumann and Zenker.

After the discovery of the crystals by Charcot and Robin in leukemia, there followed a long series of reports of their occurrence in this affection, by Vulpian, White, Mosler, Riegel, Neumann, Zenker, etc., etc. According to all these observers, the crystals are not found preformed, but they begin to appear after the blood or tissues have stood for a short time. Zenker and Neumann stated that the most striking feature in their blood-preparations was the close relationship that seemed to exist between the crystals and the white blood-cells. This was, of course, before the days of the Ehrlich stain and the differentiation thereby of the white blood-cells into different classes, but later work has shown that the cells for which the crystals evidence such an affinity are the eosinophiles, the cells with  $\alpha$ -granulation of Ehrlich. Neumann, in his later observations on this subject, in 1889, has shown that it is only in that form of leukemia in which the bone-marrow is affected, *i. e.*, the spleno-myelogenous form, that the crystals are found, and he regards it as probable that the bone-marrow cells (many of which are eosinophiles) contain the chemic constituents necessary for the crystal-formation, although Zenker and Westphal think the spleen also plays some part in this process.

Coincidently with the many observations of the crystals in leukemia, numerous articles appeared from time to time showing that the crystals are not peculiar to this disease, but also found under other conditions. Again, to Charcot do we owe our first knowledge on this subject, he, in 1856, noting their presence in a case of dry catarrh with emphysema. Bizzozero and Friedreich observed them in the sputum in bronchial catarrh, and

Förster, Zenker and Harting in bronchitis. This was the status of affairs when, in 1872, a great impetus was given to the whole subject by Leyden's report of the finding of great numbers of the crystals in the sputum in bronchial asthma, he finding them in all parts of the sputum, but especially in the little greenish-yellow plugs, made up of mucus and round cells with large refractive granules (demonstrated later by Lewy, Seifert, and others, to be eosinophiles). From their constant presence in this disease, Leyden advanced the theory that these "spitzenförmige Bösewichter" might be the cause of the asthmatic attack through mechanical or chemic irritation of the bronchial mucous membrane, causing bronchial spasm, either directly or reflexly. The observations of Leyden stimulated much work in this direction, and in all respects his observations were substantiated. Lewy showed in 26, Ungar in 30 cases, the constant presence of the crystals, the former noting their presence just before, during, and for from two to three days after the attack ; the latter calling especial attention to the close relationship existing between the eosinophiles and the crystals. Fink has recently shown that over 80% of the cells in the sputum in bronchial asthma are eosinophiles, while he, Seifert, Gollasch, Gabritschewsky, Müller, and others have shown a marked increase of these cells in the blood during the asthmatic paroxysm.

Although Curshmann regards his "spirals" as the cause of the dyspnea by blocking up the bronchioles, and Lewy and Pel regard all these substances—crystals, spirals and eosinophiles—as by-products of a primary cause, an exudative bronchopneumonia ; nevertheless Leyden, in 1891, again brought forward his original theory, following the work of Lazarus, showing the reflex nature of the asthmatic attack.

Although the crystals have been described in a few

cases of acute and chronic bronchitis, and by Naunyn and Lewy in four cases of pulmonary tuberculosis, nevertheless their presence in these conditions is so very occasional, that their appearance in the sputum may be regarded as almost pathognomonic of bronchial asthma.

Voltolini showed as early as 1871, a clinical relationship between nasal polyps and bronchial asthma, being substantiated by Lewy, Vierordt, and others; while Scheinmann has shown that by the removal of nasal polyps, asthmatic attacks have been stopped. Lewy, turning his attention to this new source of the crystals, obtained them in 32 of 47 cases of nasal polyps; in 17 of 29 in those who had not bronchial asthma, and in 15 of 18 in those affected with this disease, the crystals not being preformed, but appearing on standing. Seifert in his cases reports the presence of large numbers of eosinophiles as well.

As regards the presence of the crystals in the feces, their first observation was made by Bizzozero in a severe anemia, associated with the presence of the *dochmias duodenalis* in the intestines. It is to Perroncito and to Leichtenstern, however, that we owe most of our knowledge upon this subject, the former demonstrating their presence in the feces in association with the *anchylostomum* and the *anguillula*; the latter in almost all of his 272 cases of *anchylostomiasis*, in all cases in which the *anguillula* was found, and in many instances of intestinal disease due to other entozoa—the *oxyuris vermicularis*, *tricocephalus*, *taenia saginata*, *taenia solium*, and *ascaris lumbricoides*, and he regarded the crystals as absolutely indicative of the presence of entozoa, continuing the treatment until they are no longer found in the stools. He has shown that the crystals are found both before and after death only in those portions of the intestines occupied by the worms, and he thinks

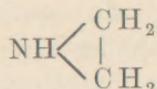
more careful examination would have demonstrated the presence of entozoa in all cases in which the crystals have been found in the feces, notably in some cases of typhoid fever reported by Bäumler and Nothnagel. Many eosinophiles are often seen in the feces in all these cases, while a marked eosinophilia has been described in the blood of patients suffering with ancylostomiasis by Zappert and others.

Beside the foregoing findings of Leichtenstern and Perroncito, a few observations of rarer associations between crystals and parasites have been noted—by Leichtenstern in the sputum in 3 cases of echinococcosis disease of the lungs; by Grawitz in a purulent pleural exudate in all probability entozoic in origin; by Israel in a small cyst formed by the pentastomum denticulatum in the liver and by Yamagiwa in the sputum in the lung-distome disease of Japan.

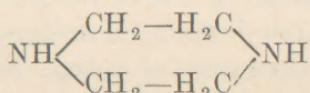
Up to 20 years ago, the chemistry of the Charcot-Leyden crystals was an unsolved problem, each observer advancing suggestions, no one offering chemic facts. Charcot thought they were proteid in nature, Robin that they were magnesium phosphate, Harting, calcium phosphate from morphologic resemblances, Kühne that they were related to vitellin, Hoppe-Seyler to the aleuron crystals of plants, while Zenker and Friedreich regarded them as tyrosin, and Salkowski and Leyden as a crystallized, mucin-like body. This was the chaotic state of affairs that greeted Schreiner, when he took up the subject in 1878. As, unfortunately, crystals from the sputum or from leukemic blood were not available in sufficient quantity, he worked with the crystals obtained by Böttcher in 1866, on drying human semen, as well as upon the surface of old pathologic specimens preserved in alcohol, and from egg-albumin. These crystals showed the same micro-chemic reactions as the Charcot-Leyden crystals, were of almost, though

not quite, the same form, and in all respects the resemblance was so striking that the two forms were regarded as identical. By exceedingly brilliant work, Schreiner showed that these crystals were the phosphoric-acid salt of a base,  $C_2H_5N$ , which he called spermin.

Thus, the matter of the chemistry of the Charcot-Leyden crystals was regarded as settled, until the whole subject was reopened at the tenth international medical congress. A few years before this congress, Brown-Séquard and Tarchanov had obtained a powerful principle from the testicles of animals, the famous elixir, and to this were ascribed the most wonderful tonic and strengthening properties, marvelous cures being reported as having been effected by it in tabes dorsalis, malaria, and all conditions of asthenia and anemia. It was from this substance that Pöhl isolated crystals identical with those of Böttcher, and it was his belief that these were the active principle of the elixir that stimulated further chemic research in this direction. Schering prepared synthetically a substance identical with Schreiner's base, which he called piperazin, or piperazidin; Ladenburg and Abel showed that the base  $C_2H_5N$  was in reality ethylenamin



while Hoffmann, Majert and Schmidt considered that it should have the double formula  $C_4H_{10}N_2$ , and that it was diethylendiamin



The Brown-Séquard elixir has gone the way of most of the royal roads to health, but Dr. Pöhl still clings to the spermin-crystals as powerful stimulants, regard-

ing their tonic action as largely due to the important rôle they play in intraorganic oxidation. He has obtained the crystals in small quantities from most of the organs, although most abundantly from the prostatic secretion, and he regards the spermin and Charcot-Leyden crystals as identical, claiming to have converted one form of crystal into the other, and assigning to each the formula  $C_5H_{14}N_2$  or some polymer. Nevertheless, the slight difference in crystallographic form, as insisted upon by Guttmann and Fürbringer, and recently demonstrated by Theodor Cohn, leaves us in some doubt regarding the question, and although the two forms of crystals are regarded by the majority of observers as identical or at least extremely closely related, further work will be necessary to settle the matter definitely.

Thus, it will be seen that the Charcot-Leyden crystals are found in the most diverse situations, in the sputum in bronchial asthma, in the feces in diseases due to intestinal parasites, in nasal polyps, in the blood and organs in leukemia and in normal bone-marrow; and the sole thread that binds together these diverse appearances is the constant association that exists between the crystals and the eosinophilic cells. The preponderance of eosinophiles in the sputum and their increase in the blood in bronchial asthma; the presence of crystals only in those forms of leukemia that show an increase of the eosinophiles in the blood; the finding of the crystals in the normal bone-marrow, which is considered to be one of the seats of origin of the eosinophiles; the increase of eosinophiles in the nasal secretion in some cases of polyp; the association of crystals and eosinophiles in the feces in intestinal diseases due to entozoa; the repeatedly made observations of the close arrangement of crystals and cells, and their identity in affinity towards the acid stains—

all point to a close relationship between them. That something beside the presence of the cells, however, is necessary for the crystal-formation is shown by a series of experiments carried on by me in the summer of 1897. The source of the eosinophiles in these experiments was the blood of a patient suffering with trichinosis. In this disease, as I have reported elsewhere, there is a marked increase in the eosinophilic cells in the blood, reaching in the case studied at that time 68.2% of all the white blood-cells, with a leukocytosis of from 20,000 to 30,000, thus furnishing an abundance of these cells. On nine occasions blood was withdrawn from the patient, some placed in sterilized, some in unsterilized vessels, some kept in the thermostat at body-temperature, some kept at room-temperature, *i.e.*, it was tested under practically all conditions.

The blood under each of these conditions was examined carefully—very frequently at first, daily afterward for a period of several weeks, but under no circumstances in any of the preparations were the Charcot-Leyden crystals found; thus demonstrating that they are not *direct* crystallization-products of the eosinophiles. With these results, the experiments carried on by Müller entirely agree, he obtaining his eosinophilic cells from the contents of pemphigus-vesicles, where they are found in abundance. What, then, is this relationship, it is impossible to say; whether the crystals are formed in the lymph-spaces of the bronchial mucous membranes, as suggested by Leyden and Weiss; whether the eosinophiles are drawn from the blood by a positive chemiotaxis, which Müller thinks the crystals possess toward them; whether cells and crystals are formed *in situ*, as Gollasch and Seifert think probable; or whether the whole process, formation of cells and crystals, is but a secretion-neurosis, as believed by Neusser—all these questions must for the

present remain unanswered. But one thing can settle the matter definitely, and that is the clearing up of the chemistry of the cell-granulations, and it is from this side, and this side alone, that we can look for further progress in the matter. Unfortunately, even our modern chemic methods are not capable of the refinements necessary to such a study, and the Ehrlich stains, although helping us wonderfully in cell-differentiation, yield us practically no information regarding the chemistry of the cell-granulation.

Perhaps in the near future, the chemistry of these microscopic particles will be a possibility; but until then we shall be compelled to say of this, the most interesting question connected with the Charcot-Leyden crystals, as for the same reason we must say of many greater and weightier medical problems, "*non liquet.*"